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PATENT
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Moya Kinnealey

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Jen Sheen

Art Unit: 1649

Serial No.: 08/989,881

Examiner: O. Zaghmout

Filed: December 12, 1997

Title: STRESS-PROTECTED TRANSGENIC PLANTS

Director of Patents
Washington, D.C. 20231

DECLARATION OF JEN SHEEN

I declare:

1. I am the inventor of the subject matter described and claimed in the above-captioned patent application.

2. I am a Molecular Biologist at Harvard Medical School in the Department of Molecular Biology at Massachusetts General Hospital. I am an author of over 50 publications in the fields of plant genetics and molecular biology, and I have served on a number of editorial/scientific advisory boards including *Plant Molecular Biology*, *Trends in Plant Science*, *The Plant Journal*, and NSF Plant Genomic Initiative.

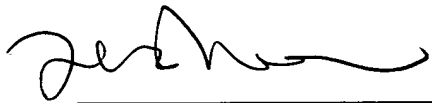
3. As outlined in the patent application, I have cloned the protein kinase (PK) domain of AtCDPK1 under the control of the 35S promoter (see pages 16-19). As depicted in Fig. 3C of the application, the PK domain was tagged with the double-hemagglutinin (DHA) epitope to follow its expression. This expression construct was introduced into wild-type *Arabidopsis* using standard *Agrobacterium*-mediated transformation methods known when the provisional patent application was filed in 1996. Four transgenic lines were subsequently selected for expression analysis. These transgenic plants showed high constitutive expression of the transgene by RT-PCR analysis. These lines were then evaluated for drought tolerance as follows. Plants (non-transformed wild-type and transgenic) were grown for three weeks under standard laboratory conditions in a growth chamber. Water was then withheld for 10 days. All four lines were found to be drought tolerant. For example, as shown below, wild-type plants (left) wilted, but transgenic plants (right) constitutively expressing the protein kinase domain of AtCDPK1 failed to wilt and remained healthy.



Based on these data, I have concluded that transgenic plants constitutively expressing a polypeptide that includes a PK domain are drought tolerant when compared to corresponding non-transformed plants.

4. All statements made herein of my knowledge are true and all statements made on information and belief are believed to be true; and further these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date 4/13/00



Jen Sheen, Ph.D.

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